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MINIREVIEW

Amphiphilic Block Copolymers for Drug Delivery

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ABSTRACT: Amphiphilic block copolymers (ABCs) have been used extensively in pharmaceutical applications ranging from sustained-release technologies to gene delivery. The utility of ABCs for delivery of therapeutic agents results from their unique chemical composition, which is characterized by a hydrophilic block that is chemically tethered to a hydrophobic block. In aqueous solution, polymeric micelles are formed via the association of ABCs into nanoscopic core/shell structures at or above the critical micelle concentration. Upon micellization, the hydrophobic core regions serve as reservoirs for hydrophobic drugs, which may be loaded by chemical, physical, or electrostatic means, depending on the specific functionalities of the core-forming block and the solubilizate. Although the Pluronics®, composed of poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide), are the most widely studied ABC system, copolymers containing poly(L-amino acid) and poly(ester) hydrophobic blocks have also shown great promise in delivery applications. Because each ABC has unique advantages with respect to drug delivery, it may be possible to choose appropriate block copolymers for specific purposes, such as prolonging circulation time, introduction of targeting moieties, and modification of the drug-release profile. ABCs have been used for numerous pharmaceutical applications including drug solubilization/stabilization, alteration of the pharmacokinetic profile of encapsulated substances, and suppression of multidrug resistance. The purpose of this minireview is to provide a concise, yet detailed, introduction to the use of ABCs and polymeric micelles as delivery agents as well as to highlight current and past work in this area. J Pharm Sci 92:1343–1355, 2003

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INTRODUCTION

Many important therapeutic compounds exhibit poor aqueous solubility, rendering delivery of those agents quite challenging. The development of effective delivery systems is crucial to the success of future drugs, which may include larger and more sophisticated synthetic compounds as well as complex natural molecules.[1]

In addition to standard formulation techniques, polymeric micelles may be used for solubilization, stabilization, and delivery of challenging agents. The functional properties of micelles based on amphiphilic block copolymers (ABCs) render them ideal for encapsulation and delivery of

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hydrophobic drugs. ABCs consist of at least two regions of distinct chemical nature that undergo phase separation as a result of chain association in solvents that selectively dissolve one of the blocks. This process results in the formation of nanoscopic supramolecular core/shell structures, i.e., polymeric micelles. The assembly of ABCs into micelles can be largely explained by the relative interactions between the hydrophilic and hydrophobic blocks with one another and the surrounding medium. During the micellization process, the hydrophobic blocks associate to form the core region, whereas the hydrophilic segments position between the core and the external aqueous medium. Hence, the hydrophobic core is stabilized by the hydrophilic shell, which serves as an interface between the bulk aqueous phase and the hydrophobic domain (Fig. 1). This unique architecture enables polymeric micelles to serve as nanoscopic depots or stabilizers for poorly water-soluble compounds.

There has been great interest in the use of polymeric micelles as drug carriers.[2–4] This minireview will begin with an outline of the fundamental principles and basic characteristics of ABC delivery vehicles. The functional aspects of the hydrophilic shell-forming blocks and micellar dimensions will be outlined briefly. Emphasis will be given to three explicit categories of poly(ethylene oxide)-based ABCs, which are classified according to the chemical nature of the core-forming hydrophobic blocks. Therefore, the ABCs will be grouped as poly(ethylene oxide)-*block*-poly(L-amino acid)s, poly(ethylene oxide)-*block*-poly(ester)s, or poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide), i.e., the Pluronics®. Although the following discussion is focused primarily on self-associated delivery systems, other closely related work and the potential of ABC unimers will also be addressed. Past work, such as that involving the delivery and/or solubilization of challenging molecules, and more current efforts, such as those focused on gene delivery, will be highlighted.

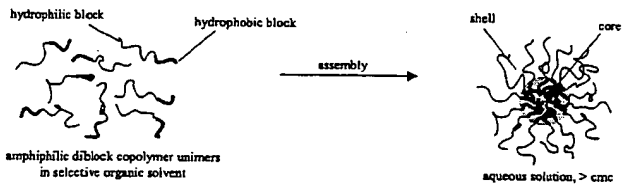

Figure 1. Micellization model for an amphiphilic AB-diblock copolymer.

GENERAL CONSIDERATIONS AND PROPERTIES

Assembly

Polymeric micelles are association colloids. Hence, they are similar in some respects to standard surfactant micelles. Both ABCs and low-molecular-weight surfactants form micellar structures at or above a threshold critical micelle concentration (CMC). Below the CMC, the number of amphiphilic molecules adsorbing at the air–water interface increases with increasing concentration. At the CMC, both the bulk solution and the interface are saturated with unimers. For both types of amphiphiles, chain association is entropically driven via the expulsion of ordered water molecules into the bulk aqueous phase. The standard change in free energy for the micellization process, ΔG°_{mic}, is described by eq. 1:

$$\Delta G^{\circ}_{mic} = RT \ln(\mathrm{CMC}) \qquad (1)$$

where R represents the gas constant and T is the temperature of the system. The CMCs of ABCs are typically on the order of 10^{6}–10^{7} M,[5,6] whereas those of low-molecular-weight surfactants are on the order of 10^{3}–10^{4} M.[7,8] For this reason, micelles formed from ABCs are generally more thermodynamically stable than those formed from low-molecular-weight surfactants. Increased thermodynamic stability indicates that polymeric micelles should be less prone to disassembly at low concentrations than standard surfactants. ABC micelles also possess kinetic stability in that the dissociation of micelle structures into free-chain unimers is a slow process, even when the system is subjected to extreme dilution.[9,10] Stability toward dilution is of particular concern for any association-based carrier system intended for parenteral administration and has implications for drug release. For example, a less thermodynamically and kinetically stable carrier might release drug prematurely because of dissociation of the micelle structure, whereas a more stable system might be preferred for long-circulating, sustained-release delivery systems.

Stealth Properties of Polymeric Micelles

A major barrier for colloidal drug carriers is nonspecific uptake by the reticuloendothelial system (RES). The RES, or mononuclear phagocyte system, is a class of cells, including monocytes and

macrophages, responsible for engulfing and clearing old cells, miscellaneous cellular debris, foreign substances, and pathogens from the bloodstream. The ability to avoid RES uptake is crucial for achieving prolonged residence time in the blood compartment. For polymeric micelles, RES uptake is primarily a function of the hydrophilic shell, which is in direct contact with blood components after parenteral administration. The most commonly used hydrophilic block for polymeric micelle drug-delivery systems is poly(ethylene oxide)/poly(ethylene glycol), PEO/PEG. In the biomaterials literature, PEO is generally used in reference to polymers with a molecular weight >10,000 g/mol, whereas PEG is usually reserved for its lower molecular weight structural equivalent.[11–13] Presently, no distinction between the terms will be made and the authors' original nomenclature will be adhered to when referring to specific research.

PEO is FDA-approved for parenteral administration and is widely used in a variety of biomedical and pharmaceutical applications.[14,15] One of the primary advantages of using PEO as a shell-forming material for polymeric micelles is low toxicity. For example, 10% PEG ($M_w = 4000$ g/mol) solutions have been safely administered intravenously to rats, guinea pigs, rabbits, and monkeys up to 16 g/kg.[16] In addition, PEO has long been recognized for its ability to minimize protein adsorption to surfaces.[11,13,17] Attachment of PEO chains to hydrophobic surfaces is particularly effective against protein adsorption because of the hydrophilicity and unique solution properties of PEO, including minimal interfacial free energy with water, high aqueous solubility, high mobility, and large exclusion volume.[13] Therefore, PEO attachment is often used to improve the biocompatibility of foreign materials.

In PEO-based polymeric micelle systems, the attached PEO brushes impart steric stability by physically blocking interparticle attraction between core regions and, possibly, hindering interactions between the core-forming blocks and blood components. In particular, high surface density and long PEO chain length minimize protein adsorption to hydrophobic surfaces.[17] For polymeric micelles, the length of the PEO blocks influences circulation time and uptake by the RES, with longer chains prolonging circulation time and decreasing RES uptake.[18] Because PEO brushes are relatively inert and impart "stealth" properties to polymeric micelles, encapsulation in polymeric micelles may be a viable approach to prolonging the circulation time of therapeutic agents.[18,19]

Alternative Shell-Forming Materials

There has been some interest in developing other hydrophilic shell-forming polymers as PEO alternatives for polymeric micelles and liposomal systems.[20,21] For example, Stepanek et al.[22] have explored the use of mixed PEO/poly(electrolyte) block copolymers as shell-forming materials for polymeric micelles. In this system, it is possible to control micelle size via the relative expansion of the shell region, which is sensitive to changes in both pH and ionic strength. Furthermore, Roux et al.[23] recently demonstrated the effectiveness of a pH-sensitive *N*-isopropylacrylamide copolymer for steric stabilization of liposomes. The use of these types of hydrophilic polymers may have a more predominant role in polymeric micelle systems in the future. PEO alternatives may ultimately allow for tailoring of the shell properties, thereby increasing the utility of polymeric micelles for less traditional delivery applications, such as targeting, bioadhesion, responsiveness to proteins/cells, and pH sensitivity.

Micellar Dimensions

Any system intended for long circulation should be small enough so that it is not removed by filtration in the capillary beds.[24] Because polymeric micelles are usually <100 nm in diameter, they should be able to pass through microvasculature. The ability to avoid renal excretion may also have a role in prolonging blood residence time of polymeric micelles. Although the renal filtration limit has been explored most thoroughly for proteins and water-soluble polymers, the principles established in those realms are applicable to polymeric micelle systems. For proteins, there is some variation in the glomerular filtration limit because of charge, shape, etc. However, the cutoff is approximately 70,000 g/mol.[25] In most cases, the molecular weight of polymeric micelles is on the order of 10^6 g/mol.[26–28] Therefore, characteristically high-molecular-weight polymeric micelles should avoid renal clearance. Conversely, ABC unimers, which fall below the glomerular filtration limit in terms of molecular weight, might be excreted upon micelle dissociation.

Generally, tumor vasculature is characterized by high permeability and decreased or defective

lymphatic drainage.[29] Consequently, it is possible for polymeric micelles, proteins, and other macromolecules to extravasate from leaky capillary beds *in vivo*, resulting in accumulation in pathological tissues that have poor lymphatic drainage.[29,30] Depending on the tumor model, the endothelial pores of tumor vasculature vary in size from approximately 100 to 1000 nm.[31] Yuan et al.[32] demonstrated that tumor vasculature is six-fold more permeable to fluorescently labeled bovine serum albumin of approximately 5 nm than to sterically stabilized liposomes of 100 nm in diameter. Furthermore, the vasculature of human tumor xenografts implanted into immunocompromised mice is permeable to sterically stabilized liposomes of up to 400 nm.[33] Hence, the nanoscopic size of polymeric micelles may impart selectivity for tumor tissues, which should help minimize harmful side effects and toxicity. The ability of macromolecules to accumulate somewhat selectively in tumor tissues has been termed the enhanced permeation and retention (EPR) effect. The EPR effect has been exploited as a strategy for altering the biodistribution of encapsulated compounds by passively targeting polymeric micelles to solid tumors.[18] An additional advantage associated with the small size of polymeric micelles is the relative ease of sterilization by filtration.

Core-Forming Blocks

In contrast to the ubiquitous use of PEO as a shell-forming material, numerous hydrophobic blocks have been studied for pharmaceutical applications. Thus, the core-forming blocks of polymeric micelle drug-delivery systems largely account for their flexibility and physicochemical properties. For clarity, the ABCs discussed in this minireview will be divided into three main categories based on the chemical structure of the core-forming block (Table 1). Specific ABC micelle systems may be further subclassified according to the method used to incorporate drugs or therapeutic biologicals. In general, the drug-loading method is determined primarily by the solubilities of the ABC and the solubilizate. Commonly used loading methods include direct dissolution,[34] dialysis,[35,36] oil/water emulsion,[37] chemical conjugation,[38] complexation,[39] and various solvent evaporation procedures.[27,40] The most commonly used methods for micelle preparation using fairly water-soluble and water-insoluble ABCs are the direct dissolution and dialysis methods, respectively. Depending on the method, drug loading may occur during or after micelle assembly. Furthermore, the manner in which drug is entrapped varies from system to system, depending on the method of loading and the ultimate goal of the research. Essentially, the mechanism of drug incorporation may be categorized as physical entrapment, chemical conjugation, or complexation. In some instances, polymer and drug may simply be administered congruently.[41] The following discussion divides PEO-based polymeric micelle systems according to the chemical nature of the core-forming block. Specific properties and advantages of each class will be addressed.

POLY(ETHYLENE OXIDE)-*BLOCK*-POLY(L-AMINO ACID)S

Polymeric micelles based on poly(ethylene oxide)-*block*-poly(L-amino acid)s, PEO-*b*-p(L-AA)s, are quite versatile because they may facilitate chemical modification to the core-forming blocks and allow loading of therapeutic substances by both chemical and physical means. The hydrophobic portions of PEO-*b*-p(L-AA)s often contain functional groups that may be derivatized to enhance the properties of the core-forming blocks with respect to drug delivery. Consequently, the p(L-AA) block may offer the possibility of post-polymerization modification by chemical means.[42]

Micelles based on poly(ethylene oxide)-*block*-poly(L-aspartate), PEO-*b*-p(L-Asp), have been used extensively for drug delivery. Because of the carboxyl functionality imparted by the p(L-Asp) block, PEO-*b*-p(L-Asp) is useful for the chemical conjugation of drugs.[18,38] When drug is loaded in this manner, the polymer/drug conjugate acts as a prodrug in that cleavage of the drug by nonspecific hydrolysis or enzymatic activity is required for drug activity. This approach is appealing for the delivery of highly cytotoxic chemotherapeutic agents. In this case, polymer/drug conjugates are assembled into micelles, enabling drug molecules to be protected in the micelle core until the carrier vehicle accumulates at solid tumor sites due to the EPR effect.[18] This strategy may minimize premature drug release and nonspecific action toward healthy cells, yet still allow for drug release in tumor tissues. Utilizing this approach with polymeric micelles may increase selectivity for tumor tissues via "passive" targeting.

Table 1. Block Copolymers Used for Drug and Gene Delivery

General Class	Incorpation Mechanism	Core-Forming Block	Encapsulated Compound	Reference
Poly(L-amino acid)	Chemical conjugation	Poly(L-aspartate)	Doxorubicin	18
		Poly(2-hydroxyethyl-L-aspartamide)	Methotrexate	38
	Physical entrapment	Poly(β-benzyl-L-aspartate)	Indomethacin	37
			KRN 5500	35
			Amphotericin B	44
			Pyrene	45
			Doxorubicin	46
		Poly(*N*-hexyl-L-aspartamide)-acyl esters	Amphotericin B	27,28,40
		Poly(β-benzyl L-glutamate)	Clonazepam	43
		Poly(C_{16}-benzyl-L-aspartate)	KRN 5500	35
		Poly(L-aspartate-DOX)	Doxorubicin	47,48
	Complexation	Poly(L-lysine)	Oligodeoxynucleotides	50
			DNA	52
		Poly(L-aspartate)	Cisplatin	39
			Lysozyme	51
		Poly(L-lysine)-succinate	Cisplatin	53
Poly(ester)	Chemical conjugation	Poly(D,L-lactic acid-*co*-glycolic acid)	Doxorubicin	69
	Physical entrapment	Poly(ε-caprolactone)	Indomethacin	6,58
			Dihydrotestosterone	36
			FK506	59
			L-685,818	59
			Nimodipine	60
		Poly(D,L-lactide)	Paclitaxel	63,64
			Doxorubicin	73
		Poly(D,L-lactic acid-*co*-glycolic acid)	Doxorubicin	69
Pluronics®	Physical entrapment	n/a (P105)	Ruboxyl	82
			Doxorubicin	82,83
		n/a (P85)	Daunorubicin	34
			Doxorubicin	34,78,81
			Vinblastine	34
			Mitomycin C	34
			Methotrexate	34
			Cisplatin	34
			Epirubicin	34
			Fluorescein	81
			Taxol	81
			Etoposide	81
			3′-azido-3′deoxythymidin	81
			Valproic acid	81

(*Continued*)

Table 1. (*Continued*)

General Class	Incorpation Mechanism	Core-Forming Block	Encapsulated Compound	Reference
			Loperamide	81
		n/a (F127)	Nystatin	86
		n/a (F68)	Nystatin	86
	Cotreatment	n/a (P85)	Digoxin	41
			Rhodamine-123	41,80
			Fluorescein	81
			Doxorubicin	81
			Taxol	81
			Etoposide	81
			3'-azido-3'deoxythymidin	81
			Valproic acid	81
			Loperamide	81

Micelles based on PEO-*b*-p(L-Asp) have also been used for the physical entrapment of drugs. In this case, drug molecules may partition out of the micellar structure and assert pharmacological activity. Loading of amphotericin B (AmB), a potent antifungal agent, into PEO-*b*-p(*N*-hexyl-stearate-L-aspartamide) micelles results in reduced hemolytic activity compared with free AmB.[40] Furthermore, polymeric micelles based on PEO-*b*-poly(*N*-hexyl-L-aspartamide)-acyl esters solubilize AmB at high concentrations and result in sustained release of encapsulated drug *in vitro*.[27,28] In one of the last examples, reconstitutable solid polymeric micelle formulations were prepared despite the relatively large size of the core-forming block.[27] The benzyl esters of both PEO-*b*-p(L-Asp) and PEO-*b*-(L-glutamate) also form micelles capable of encapsulating hydrophobic compounds and therapeutic agents.[35,43–45] For instance, indomethacin, a poorly water-soluble drug, has been loaded into poly(ethylene oxide)-*block*-poly(β-benzyl-L-aspartate), PEO-*b*-PBLA.[37] It may be possible to use PEO-*b*-PBLA to take advantage of the EPR effect. To this end, Kataoka et al.[46] have loaded doxorubicin (DOX), a potent anticancer agent, into micelles prepared from PEO-*b*-PBLA.

Micelles prepared from derivatives of PEO-*b*-PBLA have also shown promise as delivery vehicles. For example, PEG-*b*-p(C_{16}-benzyl-L-aspartate) was prepared via an ester exchange reaction between PEO-*b*-PBLA and cetyl alcohol, and then used for incorporation of a water-insoluble anticancer drug.[35] To improve core/drug compatibility, it may even be possible to chemically tether the hydrophobic block of PEO-*b*-p(L-Asp) and other functional PEO-*b*-p(L-AA)s with the drug itself. To this end, Yokoyama et al.[47] derivatized PEO-*b*-p(L-Asp) via chemical conjugation of DOX and then loaded free DOX into micelles prepared from PEO-*b*-p(L-Asp-DOX). In this system, micelles containing high quantities of both chemically attached and physically loaded DOX express potent antitumor activity against a murine colon adenocarcinoma (C26) solid tumor model.[48] In a number of studies, the chemical structure of PEO-*b*-p(L-AA) has proven important in terms of drug-loading capacity, release characteristics, and drug activity. Thus, the ability to customize the core-forming block of functional PEO-*b*-p(L-AA)s is clearly advantageous.

Some PEO-*b*-p(L-AA)s also allow for ionic interactions between polymer chains. Therefore, it is

possible to prepare polyion complex (PIC) micelles, which contain charged polymer blocks, from block copolymers containing ionized p(L-AA) core-forming blocks. For instance, mixing oppositely charged polymers, such as negatively charged PEO-*b*-p(L-Asp) and positively charged PEO-*b*-p(L-Lysine), PEO-*b*-p(L-Lys), results in the formation of PIC micelles.[49] Similarly, it may be possible to take advantage of ionic interactions between therapeutic agents and polymers. For example, complexes formed between PEG-*b*-p(L-Lys) and antisense-oligodeoxynucleotides assemble into micellar structures with core/shell architecture.[50] PIC micelles may ultimately prove useful for antisense therapy or entrapment of enzymes.[50,51]

Di- and triblock copolymers based on PEG-*b*-p(L-Lys) are currently being explored for gene delivery approaches focusing on nonviral transfection. Although micelles were not prepared, PEGylated linear, dendritic, and branched p(L-Lys) as well as PEG-grafted p(L-Lys) have been complexed with DNA and structure/activity relationships explored with respect to transfection efficiency.[52] In this case, the physicochemical properties did not correlate with *in vitro* biological activity. However, such structure/property studies may aid researchers in developing block copolymer systems for use in gene delivery. Although delivery of DNA via complexation with PEG-*b*-p(L-Lys) has not yet been successful, exploitation of ionic interactions has shown promise as a means of drug loading. For example, metal complex formation can be utilized for loading cisplatin into PEO-*b*-p(L-Asp) micelles.[39] Cisplatin-loaded methoxy PEO-*b*-p(L-Lys)-succinate releases cisplatin slowly *in vitro* and alters the biodistribution of the drug.[53]

An advantage of poly(L-AA)s is that they may be biodegradable and relatively nontoxic. The poly(amide) backbone of poly(L-AA)s may undergo hydrolysis and/or enzymatic degradation to yield biocompatible materials based on naturally occurring L-AAs. However, few toxicity/biocompatibility studies have been performed on PEO-*b*-p(L-AA)s and biodegradability remains to be established. Nonetheless, phase I clinical trials conducted in Japan suggest that DOX loaded into PEO-*b*-p(L-Asp) micelles containing a chemically conjugated DOX side chain is safe, with no overt signs of cardiotoxicity, liver toxicity, or untoward infusion-related side effects.[54] PEO-*b*-p(L-AA) systems have recently been reviewed in detail.[55]

POLY(ETHYLENE OXIDE)-*BLOCK*-POLY(ESTER)S

ABCs containing poly(ester) core-forming blocks are often used for biomaterials and drug delivery applications. Although generally less suitable for chemical modification than poly(L-AA)s, poly(esters) have a history of safe application in humans. Aliphatic poly(ester)s are susceptible to nonspecific hydrolysis, and, therefore, subject to degradation in aqueous media[56,57] For delivery purposes, some of the most commonly used poly(ester)s include poly(glycolic acid), poly(D-lactic acid), poly(D,L-lactic acid), copolymers of lactide/glycolide, and poly(ε-caprolactone).

Although the core-forming block of PEO-*b*-p(ε-caprolactone), PEO-*b*-PCL, is semicrystalline, it is possible to control the degree of crystallinity of PCL by altering the ratio of PEO to PCL.[58] Allen et al.[59] have explored this system for formulation of immunosuppressive agents and demonstrated *in vitro* biocompatibility. In addition, dihydrotestosterone is effectively solubilized by PEO-*b*-PCL micelles and retains biological activity *in vitro*.[36] PEO-*b*-PCL micelles have also been used for encapsulation of indomethacin and result in sustained *in vitro* release.[6] Ge et al.[60] demonstrated that triblock copolymers composed of PCL-*b*-PEO-*b*-PCL associate into micelle structures capable of encapsulating nimodipine, a lipophilic drug, in good yield.

Similar to micelles composed of PEO-*b*-p(L-AA)s, PEO-*b*-poly(ester) micelles may act as long-circulating drug carriers and allow for passive accumulation in tumor tissues via the EPR effect. Recently, the pharmacokinetic profile and biodistribution of PEO-*b*-PCL and PEO-*b*-PDLLA micelles have been investigated.[61,62] Altering the pharmacokinetic profile of encapsulated agents is critical for exploiting the EPR effect as a means of targeting tumor tissues. PEO-*b*-poly(ester)s have been used extensively for solubilization of paclitaxel, a poorly water-soluble and potent anticancer agent. Methoxypoly(ethylene glycol)-*b*-poly(D,L-lactide), designated MePEG-*b*-PDLLA where Me represents the methoxy end-cap group of the PEG chain, is capable of solubilizing paclitaxel at >5000 times the saturation solubility of the drug in water.[63] Whereas MePEG-*b*-PDLLA micelles are nontoxic *in vivo*, MePEG-*b*-PDLLA unimers increase the apparent solubility of paclitaxel even below the CMC of the polymer.[63,64] The basic and biological characteristics of poly(ether)-*b*-poly(ester) micelles for

formulation of paclitaxel have been reviewed by Liggins and Burt.[65]

PEO-*b*-poly(ester)s have been used for other site-specific delivery applications. For example, PEG-*b*-PDLLA has recently been functionalized with aldehyde groups at the end of the PEG chains in order to introduce surface charge and amino acid residues to the micelle exterior.[66] In this way, it is possible to increase selectivity and further alter the biodistribution of polymeric micelles via the introduction of targeting moieties, such as sugars, to the periphery of the micelle shell.[67] With the PEG-*b*-PDLLA micelle system, Nagasaki et al.[68] conjugated various sugar molecules to the shell exterior and demonstrated selective protein interaction using affinity chromatography. The introduction of sugar moieties to the exteriors of polymeric micelle drug-delivery systems may ultimately allow for active targeting via glycoreceptors. Additionally, the thermal properties of PEG-*b*-PDLLA micelles have been exploited for drug delivery.[26]

With PEO-*b*-poly(ester) micelle systems, it is possible to load drugs by both chemical and physical means. For example, PEG-*b*-poly(D,L-lactic acid-*co*-glycolic acid), PEG-*b*-PGLA, has been used for formulation of DOX through both drug conjugation and physical entrapment.[69] With this system, Yoo and Park[69] demonstrated different release profiles and postulated enhanced uptake of the drug conjugate. This system differs from those based on PEO-*b*-p(L-AA)s in that each polymer chain contains only one functional group at the end of the PGLA blocks for conjugation with DOX. Ultimately, this feature of PEG-*b*-PGLA may limit the attainable dose in animal models and humans.

In addition to their role as a core-forming material in traditional polymeric micelles, poly(esters) have often been incorporated into triblock copolymers for various delivery applications. In particular, poly(ester)s are often used in gel-forming systems. For example, triblock copolymers based on PEG-*b*-PGLA-*b*-PEG form biodegradable thermosensitive micelles.[70] With this system, it is possible to administer the formulation as a solution, which then gels at body temperature. Various biodegradable, poly(ester)-based gel-forming systems have been explored for delivery of therapeutic proteins.[56,71] Recently, Kissel et al.[72] have reviewed in detail the use of PEO-*b*-poly(ester)-*b*-PEO hydrogels for protein delivery. Similar triblock copolymers have been used for delivery of small molecules. For example, Liu et al.[73] have prepared triblock copolymer micelles based on PDLLA-*b*-PEG-*b*-PDLLA for formulation of DOX and demonstrated sustained release characteristics *in vitro*.

POLY(ETHYLENE OXIDE)-*BLOCK*-POLY(PROPYLENE OXIDE)-*BLOCK*-POLY(ETHYLENE OXIDE)

Another important class of ABCs used in drug delivery applications is the Pluronics®, also known as poloxamers. Pluronics® are triblock ABA-type copolymers based on poly(ethylene oxide)-*block*-poly(propylene oxide)-*block*-poly(ethylene oxide), which are typically expressed as $PEO_{m/2}$-*b*-PPO_n-*b*-$PEO_{m/2}$, where m and n designate the total average number of PEO and PPO repeat units, respectively. At low concentrations, Pluronics® alone are not cytotoxic.[34] A particularly attractive feature of the Pluronics® is that they are commercially available in a wide range of molecular weights and block ratios. The chemical structure of the copolymer, particularly the size of the PPO block, impacts both the CMC and partitioning of hydrophobic molecules into Pluronic® micelles.[74] Consequently, specific Pluronics® can be chosen so as to enhance drug-delivery properties.

Pluronics® have been shown to inhibit the efflux actions of P-glycoproteins (P-GP).[34,75] The overexpression of P-GP in cancerous tissues is often linked to reduced accumulation of therapeutic agents at tumor sites.[75] P-GP-mediated multidrug resistance (MDR) is a mechanism through which tumors can become resistant to a variety of functionally and structurally diverse chemotherapies.[76] Pluronics® have shown potential for suppression of MDR. Specifically, the effect of Pluronics® on P-GP-mediated efflux is mediated primarily by the unimers.[77] The sensitization effect in resistant cells appears to be related to reduced adenosine 5′-triphosphate (ATP) levels in MDR phenotype cells, i.e., energy depletion.[78] Numerous cytotoxic agents, including both MDR- and non-MDR-type drugs, have been loaded into Pluronic® micelles (Table 1).[34,77]

The use of Pluronics® may be a promising strategy for overcoming challenging drug delivery barriers, including MDR and the blood-brain barrier (BBB). The BBB is a barrier between the central nervous system (CNS) and systemic circulation, resulting from tight junctions between microvessel endothelial cells. Furthermore, efflux proteins decrease transcellular transport of

therapeutic compounds across the BBB. These characteristics make the BBB a substantial obstacle to CNS delivery.[79] Inhibition of the P-GP efflux transport system by Pluronics® may facilitate the delivery of therapeutic agents to the CNS.[41] Pluronic® P85 induces ATP depletion and increased membrane fluidity in bovine microvessel endothelial cells, an *in vitro* model of the BBB.[80] In addition, the coadministration of digoxin and Pluronic® P85 increases the delivery of digoxin across the BBB *in vitro* by inhibiting P-GP-mediated drug transport and enhances delivery to the brain.[41] Pluronic® P85, both above and below the CMC, has been used to increase transport of a number of therapeutic agents across a model BBB and Caco-2 cells.[81]

The use of ultrasonic enhancement has been explored as a method for controlled, or targeted, delivery with Pluronic® micelles. This approach makes use of the EPR effect to achieve selective accumulation at the target, i.e., tumor site. Then, ultrasound is applied exclusively to the tumor to yield localized drug delivery.[82] Marin et al.[83] demonstrated that ultrasound enhances drug uptake *in vitro* by increasing both micelle uptake by HL-60 cells and the concentration of free drug in the incubation medium. This effect results in increased DOX uptake, even in MDR cells.[84] Like PEO-*b*-poly(ester)-*b*-PEO, Pluronics® have been used in gelling systems. For instance, mixed Pluronic® solutions containing F127 and F68 have been used for the development of a thermosetting ophthalmic drug-delivery device in order to increase ocular residence time.[85] Pluronics® F127 and F68 have also shown promise for formulation of nystatin, a polyene macrolide antifungal agent.[86] Using Pluronics®, it might be possible to attenuate the toxicity of nystatin via modulation of the self-aggregation state of the drug. Kabanov et al.[87,88] have detailed the use of Pluronics® in both drug and gene delivery.

CONCLUSIONS

ABCs have proven useful and versatile for the delivery of therapeutic agents. In this regard, perhaps the most distinctive and functional aspect of ABCs is their ability to form micelles. Polymeric micelles are unique because of their characteristic nanoscopic core/shell architecture, and offer a viable means for the solubilization of hydrophobic compounds. Whereas the shell imparts steric stability and may possess targeting moieties, the versatility and stability of ABC micelles results primarily from the chemical composition of the hydrophobic blocks. The chemical nature of the core-forming block may allow drug incorporation via chemical, physical, and/or electrostatic means. Depending on the application and desired release profile, more or less stable delivery systems might be necessary. Characteristically, micelles formed from ABCs are particularly stable to dilution and may exhibit prolonged circulation. Because diameters of <100 nm are generally considered prerequisite for avoiding RES uptake and increasing blood residence time, the size of polymeric micelles is a key factor in determining the biodistribution of encapsulated agents. Drugs may be encapsulated, and polymeric micelles may be prepared by a variety of techniques that include, but are not limited to, direct dissolution, dialysis, chemical complexation, O/W emulsion, and various solvent removal procedures. Once loaded, drugs are protected from the physiological environment, which might prohibit nonspecific and enzymatic hydrolysis, pH-dependent degradation, etc. Furthermore, drugs may be released in a controlled manner.

With all of these considerations in mind, it is possible to choose appropriate polymers and/or tailor ABCs to the task at hand, whether that be burst or sustained release, gene delivery, prolonged circulation, targeting, bioadhesion, etc. In some instances, unimers may be desirable and administered preferentially over micelles. In addition, it might be beneficial to reformulate existing compounds to minimize toxicity, alter the pharmacokinetic profile, and, ultimately, improve the therapeutic window of potent, yet toxic, compounds. Block copolymers are beginning to have a prominent role in gene delivery and might eventually be capable of replacing the viral vectors often used for such applications. To date, most research efforts have been focused on the hydrophobic core-forming blocks of ABCs. However, there is interest in the development of new shell-forming materials, which might elicit an appropriate response to a biological trigger, such as pH changes and protein recognition. Although exploitation of the EPR effect is often used for passive targeting, active targeting may have a more important role in future delivery efforts. Perhaps the introduction of targeting moieties to the hydrophilic shell-forming blocks will become more important and prevalent as researchers continue to strive for "smart" delivery systems that might be capable of targeting specific receptors or

cell types exclusively. The development of new polymers will surely lend greater flexibility and potential to delivery systems based on ABCs. The use of ABCs is a feasible and attractive alternative to standard formulation techniques. In particular, entrapment in polymeric micelles may provide a viable approach for stabilizing substances during systemic circulation. The use of ABCs for delivery purposes will likely increase as scientists are confronted with more and more challenging compounds and biologicals.

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